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DOI

[10.1046/j.1423-0410.1997.73200971.x](https://doi.org/10.1046/j.1423-0410.1997.73200971.x)

Publication date

1997

Published in

Vox Sanguinis

[Link to publication](#)

Citation for published version (APA):

Reesink, H. W., & Engelfriet, C. P. (1997). What are the consequences of indeterminate results in confirmatory tests for antibodies against transmissible viruses ? *Vox Sanguinis*, 73, 97-104. <https://doi.org/10.1046/j.1423-0410.1997.73200971.x>

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What Are the Consequences of Indeterminate Results in Confirmatory Tests for Antibodies against Transmissible Viruses?

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In spite of considerable improvement of screening and confirmatory tests for the detection of antibodies against various viruses which can be transmitted by blood or blood products, indeterminate results are still frequently obtained in confirmatory assays. Since, as judged from the literature, there does not seem to be a general consensus on the consequences of such indeterminate results, particularly with regard to a possible reentry of the donor, we decided to devote an International Forum to this subject and the following questions were put to 15 experts in the field:

1. Which screening test(s) do you use in your country for anti-HCV and which confirmatory test(s)? What are the consequences of indeterminate results in the latter for the unit in question and for the donor. Are there follow-up investigations and are there any circumstances in which the donor can be restored to the donor panel?

2. Which screening test(s) do you use for anti-HIV and which confirmatory test(s)? What are the consequences of indeterminate results in the latter for the unit in question and for the donor. Are there follow-up investigations and are there any circumstances in which the donor can be restored to the donor panel?

3. Which screening test(s) do you use for anti-HTLV-I/II and which confirmatory

test(s)? What are the consequences of indeterminate results in the latter for the unit in question and for the donor. Are there follow-up investigations and are there any circumstances in which the donor can be restored to the donor panel?

Answers to these questions were received from 9 of the experts (see below).

Question 1

Only third generation (3.0) ELISAs are used for screening for anti-HCV except in the USA where, because only one 3.0 ELISA has been licensed by the FDA, two second generation (2.0) ELISAs are still in use. In Japan passive hemagglutination and gelatin particle agglutination tests are used apparently exclusively. For confirmation immunoblots, particularly the third generation RIBA and PCR, are used in most countries. In the USA, the second generation (2.0) Chiron Strip Immunoassay (RIBA 2.0) is the only licensed confirmation test and in Japan confirmation is based on titration in the agglutination tests. In all countries units which gave repeatedly positive results in the screening test are discarded. With regard to the main question, i.e. the consequences of positive results of screening and indeterminate results in confirmatory assays for a possible reentry of the donor into the donor panel, there clearly is no general policy. In several countries there is no reentry protocol. In the USA, a donor may reenter the panel if the confirmatory test is negative and, 6 months later, both the screening and the confirmatory tests are negative. In Germany a donor may again be asked to donate, if after a repeatedly positive screening result, an ELISA from another manufacturer, the RIBA 3.0 as well as the PCR are negative. There are no guidelines for reentry after an indeterminate result in a confirmatory assay.

In France a donor with false reactivity in the screening test can only be restored to the donor panel, when in a different assay, used as screening test a negative result is obtained.

In the UK, when the results of the screening tests are positive, reentry, on the basis of a validated alternative ELISA without further referral for confirmation, is possible when the results of a confirmatory assay are negative on two occasions, at least 6 months apart. No reentry is possible after an intermediate result in a confirmatory assay.

In The Netherlands a donor may reenter the panel if after a first sample was found to be repeatedly positive in a screening test, the same sample is negative in the RIBA 3.0. The PCR test (on a fresh sample) is only used when RIBA 3.0 is indeterminate or positive. Donors repeatedly positive in a screening test but negative in confirmatory tests are deferred for at least 2 years.

Question 2

In all countries, except Germany, testing for anti-HIV-1 as well as anti-HIV-2 is obligatory and third-generation combination ELISAs are generally applied. In the UK and France a test that can detect antisubtype O is required. In Japan gelatin particle agglutination assays are used for screening. In Australia, repeatedly reactive samples are tested in other screening assays and unless all reactions are negative, the sample is referred for confirmation. In Germany and France repeatedly reactive samples are tested in one other independent screening assay. For confirmation, the Western blot is used in all countries, whereas p24 antigen testing is done in three. A unit of blood is discarded if the screening test is repeatedly positive, irrespective of the result of the confirmation tests in the USA, Japan, the UK, France and The Netherlands. It is not

clear from some of the answers what is done with units with a repeatedly positive screening test but a negative confirmatory test. If the result of the screening test is positive and the Western blot indeterminate the plasma from later donations is used in Australia for fractionation provided the screening test has become negative. Reentry of a donor after a repeatedly positive screening result is possible in the USA if all confirmatory test results are negative and, in addition, separate ELISAs for anti-HIV-1 and anti-HIV-2 are also negative and if a sample, tested after 6 months, is negative in the screening and confirmatory tests. Testing for p24 antigen has introduced a new population of false-positive reactivities in the USA (see contribution from USA). In Australia reentry is possible if during follow-up the screening test reverts to nonreactivity. In France reentry is possible when the result of an alternative screening test on the original sample is negative. In Germany a donor may donate again, if after repeatedly positive results in a screening test, results of alternative screening tests, confirmatory assays and the PCR are all negative. In the UK and Italy donors with an indeterminate result in the confirmatory assay are permanently deferred. The UK algorithm for reentry of false positives is as for HCV. In The Netherlands a donor may reenter the panel if after a first sample was found to be repeatedly positive at screening, the same sample is found to be negative in Western blot and HIV p24 antigen tests. The PCR test (on a fresh sample) is only used when the Western blot is indeterminate and/or the p24 antigen test is positive.

Question 3

Donors are not screened for anti-HTLV-I/-II in Thailand, Italy, Germany and the UK. In the other countries, except in Japan, only anti-HTLV-I ELISAs are used but it is generally accepted that most HTLV-II antibodies are detected in this assay. In Japan a gelatin particle agglutination test is used. For confirmation Western blots are used and PCR as well in some countries. In the USA, donors with a repeatedly reactive screening test and a negative or indeterminate Western blot are permitted to donate one more time and a nonnegative result at that time leads to permanent deferral but a negative result will requalify the donor. In Australia, the cellular components from a unit with an indeterminate result are discarded but the plasma can still be used for fractionation. Positive results (in the screening test) on two subsequent donations lead to permanent deferral. In France, if the screening test is repeatedly positive, the unit is dis-

carded and two other screening tests are applied. If both are negative, the results of the first test are considered to be false. If one or two of the other two tests are positive, Western blot is done. In The Netherlands a donor may reenter the pool if, after a first sample was repeatedly positive at screening, the same sample is negative with the Western blot. The PCR test (on a fresh sample) is only used when Western blot is indeterminate or positive.

In conclusion, it is clear that there is no identical policy concerning several aspects of screening for and confirmation of antiviral antibodies and the consequences for reentry or deferral of the donor. An international consensus on this important subject would be of great value.

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Question 1

In the United States, three EIA tests are currently licensed for anti.HCV: version 2.0 level tests from Ortho and Abbott and a version 3.0 test from Ortho. However, the only licensed confirmatory test is the Chiron Strip Immunoassay (SIA), version 2.0. The FDA has provided guidance which indicates that the version 2.0 SIA cannot be used to requalify donors found reactive by the version 3.0 screening assay. The rationale is that the NS5 antigen, which is present in the screening test, is not present in the 2.0 version SIA. Since FDA-approved procedures for requalifying donors require the use of a licensed confirmatory or supplemental test, this means that, pending licensure of a 3.0 version of the SIA,

such requalification is restricted to users of 2.0 level screening and SIA tests. In these circumstances, a donor may be considered for reentry if, at the initial test, the SIA is negative and, on a subsequent sample, 6 months later, both a screening and an SIA test are negative.

With respect to product management, all blood or components must be destroyed on the basis of a repeatedly reactive screening test, irrespective of the result of a confirmatory assay (this is true for all of the tests discussed here). The donor is indefinitely deferred. Confirmatory testing for anti-HCV is not obligatory, unless an attempt is made to requalify the donor. When repeatedly reactive samples are subjected to confirmatory testing, about 15–20% are indeterminate and about 60% are confirmed positive. Donors with repeatedly reactive findings on a screening test are notified of the results of the screening and, if performed, the confirmatory tests, and are advised that they may no longer donate. Individuals with an indeterminate result are advised that the result is not definitive and are provided with information about the significance of the result and about HCV infection. Some locations perform additional testing by genome amplification, but this is not common. Any testing additional to the licensed screening and confirmatory testing may be used only for donor notification and counseling.

Question 2

Currently, all donations in the United States must be tested for anti-HIV-1 and for anti-HIV-2. There are currently only two licensed combination EIA assays, supplied by Abbott Laboratories and Genetic Systems. These are the most commonly used screening test procedures. The FDA requires that all repeatedly reactive results be subjected to 'an additional, more specific', or confirmatory test, which must also be licensed. Currently, three Western blot procedures are licensed for HIV-1 (from Cambridge Biotech through Ortho, from Biorad and from Epitope through Organon Teknika), but there are no licensed confirmatory procedures for HIV-2. In addition, an indirect fluorescence assay for anti-HIV-1 is licensed and is available from Waldheim Pharmazeutika distributed by Genetic Systems.

Donations must be discarded on the basis of a repeatedly reactive screening test and the donor must be indefinitely deferred. Present FDA guidance for reentry is complex, and requires that all confirmatory test results be negative and that, in addition, separate EIA

tests for HIV-1 and for HIV-2 also be negative. An additional sample, drawn 6 months later, must also be negative on the screening EIA and on confirmatory and other separate EIA tests. It is not permissible to collect a unit at this time, but the donor may return for a subsequent donation. The process is so complex that most agencies do not routinely undertake it. Recently, however, the FDA has discussed, but not authorized, a much simpler algorithm which, in some circumstances, will permit re-entry of individuals with an indeterminate blot pattern.

Current approved criteria for defining a blot as positive are those proposed by the CDC and ASTPHLD (Association of State and Territorial Public Health Laboratory Directors); that is, the presence of at least two of the following bands: p24, gp41 and gp120/160 [1]. These criteria are highly sensitive and have, in fact, been shown to generate a measurable number of false-positive interpretations. As a consequence, however, it is extremely unlikely that any indeterminate blot patterns (defined as any band pattern other than a positive) would be associated with actual HIV infection [2]. Nevertheless, such donors must be notified that their blot is indeterminate and cannot be defined as positive or negative. On the basis of longstanding advice from the CDC – advice which is seriously in need of update – they are advised that, if the blot pattern has not progressed after 6 months, they are ‘almost certainly not’ infected with HIV. As a matter of course, most institutions are comfortable suggesting earlier follow-up testing. An additional complication is that the FDA-required confirmatory algorithm requires that individuals with negative or indeterminate findings on an HIV-1 blot must also be tested by a licensed EIA for HIV-2 antibodies. This generates a number of reactive results which cannot be further confirmed, but which must, nevertheless, be transmitted to the donor. It is worth noting that, since the implementation of HIV-1/2 testing in 1992, only one documented HIV-2 infection has been noted among more than 50 million donations. Once again, the FDA requires that the interpretations of the licensed tests, as defined in the product insert, be transmitted to the donor. Some institutions try to reduce the frequency of indeterminate results by using the IFA test in place of the blot.

Donors with indeterminate results are advised that this is not a definitive finding and that follow-up is appropriate, they are also advised about HIV infection and its transmission and are advised to take measures to reduce this risk, pending notification. The ma-

majority of institutions do not perform additional testing on indeterminate samples, although they will frequently accept samples for follow-up evaluation, generally using routine serologic tests for this purpose.

An additional complication has been introduced as a result of testing for the HIV p24 antigen. The confirmatory test for a repeatedly reactive sample is an immunological blocking assay. However, because of concern about sample stability, the FDA will only permit the results of the confirmatory procedure to be interpreted as confirmed, indeterminate or invalid. Consequently, there is now a new population of HIV indeterminate donors, amounting to about 0.02% of all donors. These individuals must be advised of their results, but may requalify for donation if they are nonreactive for HIV antigen and anti-HIV-1 and anti-HIV-2 after 8 weeks.

Question 3

All whole blood donors must be tested for HTLV-I antibodies. Currently, three licensed tests are available, from Abbott, Cambridge Biotech (distributed by Ortho), and Organon Teknika. All are licensed only for the detection of HTLV-I, although it is accepted that each test also detects a substantial proportion of HTLV-II infections. Currently, there are no licensed confirmatory or supplementary tests for anti-HTLV-I. Nevertheless, most institutions do perform Western blot tests in order to assist in donor notification. Because counselling advice differs slightly between the two viruses, confirmatory algorithms that include viral typing are preferred. Interestingly, individuals with a repeatedly reactive screening test and a negative or indeterminate Western blot are permitted to donate one more time. A nonnegative result on this donation will lead to permanent deferral, but a negative screening test will requalify the donor.

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Question 1

The Abbott 3.0 ELISA test is used for screening purposes and for repeatedly reactive samples. The supplemental assays used are Murex HCV EIA and Monolisa HCV EIA (new antigens).

For those samples with reactivity on one or both supplemental assays a Murex Western blot is performed and interpreted according to manufacturer's instructions. Samples with reactivity on one supplemental EIA only are referred to as indeterminate regardless of HCV Western blot results. Samples with reactivity on both supplementary EIAs but HCV Western blot negative or indeterminate are also referred to as indeterminate.

Units with indeterminate reactivity are discarded and the donor is requested to return for retesting. Retests are performed on a fresh sample and include PCR testing. On completion of repeat testing, the donor is appropriately counselled and permanently deferred with reference to a gastroenterologist for clinical assessment.

No reentry protocol has currently been implemented. The above policy is consistent with national policy.

Question 2

The Genelavia MIXT (Sanofi Diagnostic Pasteur) is used for screening blood donations. Repeatedly reactive samples are subjected to supplemental testing with Abbott HIV-1/2 3rd Generation Plus, Genetic Systems HIV-2 EIA and Serodia HIV-1 Particle Agglutination Assay. Samples reactive on one or more supplemental tests are referred to a reference laboratory for Western blot.

The indeterminate unit is discarded and the donor is monitored for at least 6 months and a minimum of two donations. During follow-up, collections from such a donor have restricted usage if negative on the screening test (plasma for fractionation only). If after follow-up the screening test remains repeatedly reactive, the donor is informed, permanently deferred and referred to a general practitioner for counselling. Reentry of such a donor onto the donor panel is possible if after follow-up the screening test reverts to nonreactive status.

Question 3

Vironostika HTLV-1 (Organon Teknika) is used as a screening assay. Repeat reactive

samples are referred to a reference laboratory for supplemental tests (Cambridge HTLV-1 EIA and Serodia HTLV-1 Particle Agglutination). Samples reactive on one or both of these supplemental tests have an HTLV Western blot performed for confirmation. The cells from the indeterminate unit are discarded and the plasma is sent for fractionation. The indeterminate donor is monitored over time and the usage is restricted to plasma for fractionation. If the HTLV-I supplemental tests remain reactive on two subsequent donations over a minimum period of 6 months, the donor is informed and permanently deferred.

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Question 1

A passive hemagglutination (PHA, Dai-nabot, Japan) or a gelatin particle agglutination (PA, Ortho Clinical Diagnostics, K.K., Japan) test with fixed erythrocytes or gelatin particles coated with recombinant NS3/NS4 and core antigens is used for anti-HCV determination [1].

For screening serum is used in a 2⁵ dilution for PHA and in a 2⁴ dilution for PA. For confirmation end titer determination by 2-fold serial dilution is used. The PHA and PA titers correlate well with HCV RNA positivity as determined by PCR.

PHA $\leq 2^5$: 0/71 (0%), 2⁶⁻¹¹: 13/291 (4.4%), $\geq 2^{12}$: 36/38 (94.7%); PA $\leq 2^4$: 0/124 (0%), 2⁵⁻¹¹: 20/246 (8.1%), $\geq 2^{12}$: 29/30 (96.7%).

Follow-up investigation: for postdonation counselling, blood donors who are positive at high titer ($\geq 2^{12}$) or at low titer (PHA: 2⁵⁻¹¹, PA: 2⁴⁻¹¹) with abnormal ALT (≥ 61 IU) are notified and advised to consult a hepatologist. Because most HCV infections are persistent, all serologically positive donors are deferred from further blood donation and we do no follow-up investigations aimed at readmission to the donor panel. A clinical follow-up study of transfused patients after PHA-PA screening demonstrated that there was prevention of posttransfusion non-A/non-B hepatitis [2].

Question 2

Gelatin particle agglutination for HIV-1 and HIV-2 (Serodia HIV-1 and Serodia HIV-2, Fujirebio, Japan) is used. For screening a 2⁴ dilution of the serum is used. For confirmation the Western blot is applied.

By the end of 1995, 251 out of 70,855,969 donated blood units (0.354/10⁵) were anti-HIV-1 confirmatory positive. Serologically positive donors are deferred from further donation and no follow-up investigations are done for restoration to the donor panel positive donors. Although we have not observed any posttransfusion HIV-1 or HIV-2 infections after screening by Serodia 1 or 2 in 29,802 transfused patients [3], the two testing reagents have been combined into one test using gelatin particles coated with recombinant HIV-1/gp41, HIV-1/p24 and HIV-2/gp36 (Genedia HIV-1/2, Fujirebio, Japan) since October 1996.

Question 3

A gelatin PA test is applied for anti-HTLV-1 (Serodia HTLV-1, Fujirebio, Japan) and it has been used since 1990. For screening in the Serodia HTLV-1 serum is used in a 2³ dilution. For confirmation immunofluorescence (IFA) is used [4].

Immunofluorescence antibody positivity correlates well with positivity of provirus DNA detected by PCR in peripheral blood mononuclear cells and serum PA titer [5].

PA titer	Number tested	IFA-positive	Provirus DNA-positive
$\leq 2^3$	101	0	0
2 ⁴⁻⁸	84	18	18
$\geq 2^9$	41	41	41

PA positive donors are deferred from further blood donation and we have not done follow-up investigations for restoration of these donors to reenter them in the donor panel.

We have not carried out HTL-II screening.

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In Germany we have a group of experts nominated by the Federal Ministry of Health called 'Arbeitskreis Blut' (Working Party for Blood). In this group we have worked out a protocol for the investigation and handling of donors, positive with the enzyme immunoassays (EIA) for HIV1/2 and HCV antibodies and HB surface antigen. For HIV, HBV and HCV we have drawn up the following guidelines [1, 2]. In Germany only third generation screening tests (EIA) licensed by the Paul-Ehrlich Institute are allowed. EIA-positive samples or those with indeterminate results (i.e., in the 'grey zone') have to be retested with the same test twice. In case of negativity there is no further restriction for donor and product. In case of positivity or indeterminate results of one or both tests the donated blood has to be discarded. The positive sample has to be retested in a second EIA from another manufacturer in duplicate. Additional tests have to be performed: for HCV supplementary tests such as RIBA or matrix test, and for HIV immunoblot and p24 antigen test.

If negative or indeterminate results are obtained in the supplementary tests the PCR is recommended. If all of these tests are negative the donor can be asked for donation again. If one of the tests is positive or indeter-

minate the industry which has received plasma for plasma products from previous donations has to be informed within 7 days after donation in order to have the opportunity to block the use of plasma before final results are obtained. The donor is asked to give a second blood sample which is investigated with all tests including PCR.

If the tests of the second blood sample are all negative and missampling can be excluded the donor may be asked for further blood donation. The industry can use the plasma from previous donations. If the previous test results are confirmed the donor has to be informed and excluded from further donations. In addition a 'look-back' concerning previously donated blood products of this donor has to be started. The official regional and federal control authorities have to be informed. In case of indeterminate results additional investigations at 2- to 4-week intervals are recommended. Although not mentioned in the guidelines it is possible to restrict the expensive supplementary tests to the investigation of the second blood sample after confirmation of the positive or indeterminate results, if the donor can be reached quickly enough (within 1 week).

The guidelines say nothing about reentry of donors, which from my personal point of view should be possible for HCV after 1 year and HIV after 6 months, if only indeterminate results have been obtained which have become negative in the meantime.

Finally the 'Arbeitskreis Blut' has decided to omit any testing on HTLV-I/II at the moment because of the epidemiological situation in Germany and the quality of the available tests.

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French regulations require that each blood donation found to be repeatedly reactive by a screening test for any marker of transmissible viruses be destroyed. Results obtained with confirmation tests and possibly other investigations which are applied to these units repeatedly reactive by ELISA are only used for informing the donor.

'Indeterminate results' are not in my opinion an adequate expression since they may cover quite different situations and basically, a test result cannot be or at least cannot remain 'indeterminate'. What a donor needs is to know whether or not he/she is actually infected. If the donor is proven not to be infected, he/she is informed that on the basis of screening test results not related with an infection, his/her donations are temporarily unsuitable. This procedure is used to avoid collecting blood which will be destroyed, as long as the same screening test is used.

As nonspecific reactivity is often restricted to a single manufacturer's screening test, blood from these donors may be released for donation on the basis of a negative result using another screening test. As an 'indeterminate result' has no consequence for the unit (which has been already destroyed) and as this type of result ('indeterminate') cannot be given to the donor, the procedures to distinguish between true and false reactivities are described in the following sections.

Question 1

The screening test used is one of those registered by l'Agence du médicament: Abbott HCV EIA 3.0 (North Chicago, Ill., USA), Monolisa anti-HCV new antigens from Sanofi Diagnostics Pasteur (Marnes la Coquette, France), Murex anti-HCV version III (Dartford, UK) with a modified cutoff of N+0.4, Ortho HCV 3.0 Elisa Test System (Raritan, N.J., USA). More recently, an anti-HCV screening test on Prism (Abbott) has also been used.

Immunoblots are used as confirmatory tests. Five anti-HCV immunoblots are registered in France but the most commonly used is RIBA HCV 3.0 from Chiron (Emeryville, Calif., USA). The presence of anti-HCV antibodies is confirmed when at least two bands including bands on core or NS3 protein are reactive. The screening test result is considered false-positive when the confirmatory test result is negative or on the basis of a single band on NS4 or on NS5 proteins with the

same pattern on a subsequent sample. With an isolated core or NS3 reactivity, PCR is necessary to distinguish between presence or absence of infection (residual antibodies or false reactivity).

Question 2

French blood transfusion centers must use a combined (anti-HIV-1 + anti-HIV-2) screening test registered by l'Agence du médicament. Among the 17 registered screening tests, 5 considered as the most sensitive after the reevaluation of the screening tests in 1995 [1] were recommended by the French blood agency: HIV-1/HIV-2 plus 3rd generation (Abbott, Chicago, Ill., USA), Biotest anti-HIV-1/2 recombinant (Dreieich, Germany), Enzygnost anti-HIV-1/2 plus from Behring (Marburg, Germany), Genscreen HIV-1/2 from Sanofi Diagnostics Pasteur (Marnes la Coquette, France), Vironostika HIV Uniform II plus O from Organon Teknika (Boxtel, The Netherlands). More recently the anti-HIV screening test on Prism is also used.

Confirmation tests are mainly Western blot HIV-1. They are manufactured by Cambridge-Biotech (Worcester, Mass., USA), by Biorad (Hercules, Calif., USA), by Genelabs Diagnostics (Singapore) or by Sanofi Diagnostics Pasteur. Recently, immunoblots from Chiron and from Innogenetics (Zwijnaarde, Belgium) were considered as confirmatory tests.

A sample is confirmed positive for anti-HIV antibodies on the basis of at least 3 bands by Western blot, on gp160, gp120 and p24. A sample with a p17 band with or without gap precursor bands is considered as negative [2]. In all the other cases including a negative Western blot result, HIV-Ag testing and one or two alternative anti-HIV screening tests help in the interpretation. As false-negative Western blot results occur in the early phase of infection and as false-positive Western blot reactivities (on p24 or on gp160 or on both) are persistent over time [3], a follow-up of the donor which can be done as early as 2 weeks after the donation [4] is sometimes necessary. This follow-up allows to distinguish between true infection or false reactivity because titers of antibodies to HIV increase very quickly after infection and false reactivities remain stable. When an HIV-2 or an HIV-1 group O is suspected, specific confirmatory assays must be performed.

As mentioned earlier, a donor who was proven not to be infected by HIV cannot be restored to the donor panel if his serum remains reactive in the screening test. As this false reactivity is generally persistent from lot

to lot, the reentry of the donor will be possible only if a negative result is obtained with a different assay used as a screening test, since it is the screening test which governs.

Question 3

The screening test used is one of the 7 ELISA registered by l'Agence du médicament: HTLV-I EIA from Abbott, HTLV-I + II recombinant 3rd generation from Genelabs, Murex HTLV-I + II, HTLV-I (rgp21e enhanced) EIA from Ortho, Cobas Core anti-HTLV-I/II EIA from Roche (Basel, Switzerland), Platelia HTLV-I new from Sanofi Diagnostics Pasteur and Vironostika HTLV-I from Organon Teknika.

An ELISA which will be largely used as a screening test in the near future is the new test from Murex in a sandwich format, due to its high sensitivity and specificity. Serodia ATLA from Fujirebio (Tokyo, Japan) is generally used as a control of samples repeatedly reactive by ELISA.

Two anti-HTLV Western blots are registered in France: HTLV-I (rp21e-enhanced) Western blot from Cambridge-Biotech and HTLV blot version 2.4 from Genelabs. The latter allows to distinguish between HTLV-I and HTLV-II and has a modified rgp21 named GD21 which is more specific.

After a reactive screening test, two algorithms can be used for the interpretation: to perform 2 other screening tests including Serodia or to perform Western blot only. If the 2 screening tests give negative results, the sample is considered as negative. If one or two positive results are obtained, the Western blot is then performed. When *gag* reactivities are not associated with *env* reactivities (rgp21, gp46) no other test is necessary to conclude an absence of HTLV infection [5]. An isolated reactivity to rgp21 corresponds generally also to a false reactivity but as it can sign a recent seroconversion [6], a follow-up is recommended. In conclusion, to obtain a maximum safety of the blood supply, the protocols to discard donations from transfusion use cannot be rational. The screening test result determines in France whether to release a blood donation or not, even if we know that many ELISA reactive samples are false-positive. We also know that many ELISA-negative samples could give false reactivities by Western blot, i.e. an 'indeterminate result' and that Western blot may be negative in the early phase of HIV infection whereas ELISAs are reactive.

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Question 1

The screening test currently used in our laboratory for the detection of antibodies to hepatitis C virus in blood donors is the Ortho HCV 3.0 ELISA Test System (Ortho Diagnostic Systems, Raritan, N.J., USA). In case of positive results, samples are tested using the Chiron RIBA HCV 3.0 SIA (Ortho Diagnostic Systems). HCV RNA determination is performed by the Amplicor HCV PCR kit (Roche Molecular Systems, Basel, Switzerland) in all samples reactive to at least one antigen of the RIBA.

Units collected from donors showing positive results by the screening test are discarded, regardless of the results obtained in the

confirmatory assays, as prescribed by the Italian law.

Because RIBA-indeterminate donors may have actual infections [1, 2] and show histological findings of liver damage on liver biopsy [2, 3], we perform an HCV RNA determination in these subjects. HCV RNA-positive donors undergo a follow-up program consisting of clinical and laboratory evaluation at 1- to 3-month intervals, during which a liver biopsy is proposed [2, 3]. As regards HCV RNA negative donors, we perform a laboratory evaluation once a year.

Although we have evidence that isolated reactivity to certain antigens (i.e., anti-NS5) is not associated with viremia, in our setting donors showing such reactivity are not restored to the donor panel. In fact, in addition to legal concerns, this would require costly supplemental testing at each donation, to document the absence of viremia.

Question 2

As regards anti-HIV, the screening test currently used in our lab is the Ortho HIV-1/HIV-2 Enhanced ELISA Test System (Ortho Diagnostic Systems), while the confirmatory test is the Diagnostic Biotechnology HIV Blot 2.2 (Diagnostic Biotechnology, Singapore). According to the Italian law, units collected from donors showing an indeterminate anti-HIV pattern are discarded, and the donor is permanently deferred from donation. HIV DNA and/or HIV RNA determinations are also performed by in-house PCR techniques. Blood donors showing an indeterminate pattern are invited to enter a clinical and laboratory follow-up at 6-month intervals for at least 1 year.

Question 3

In Italy, anti-HTLV-I/II screening of blood donors is not mandatory. For research only, we use the HTLV 1+2 ELISA (Murex, Dartford, UK) and the Diagnostic Biotechnology HTLV Blot 2.4 (Diagnostic Biotechnology, Singapore).

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In the UK the national blood services perform routine virological screening of blood donors in accordance with published guidelines. The virological screening component of these guidelines is formulated in conjunction with a UK Standing Committee on Transfusion Transmitted Infections (SACTTI). The principles underlying the performance and management of all the routine virological assays are similar and are followed throughout the UK, although differences in the detail of management may occur. We have therefore provided generic responses relating to both anti-HIV and anti-HCV tests; we do not currently screen for anti-HTLV in the UK but if we were to do so, similar principles would apply.

Screening Tests

Blood centres are required to restrict their choice of manufacturer's assays (in practice, all based on ELISA) to those that have been evaluated and considered to be suitable. Separate evaluations (with exchange of information) are undertaken by the English and Scottish Blood Services and these evaluations also assist the two national plasma fractionation plants in complying with their licensing requirements. In general, assay sensitivity equivalent to 'third generation' tests is expected and for anti-HIV kits, detection of anti-HIV₁, anti-HIV₁ subtype O and anti-HIV₂ is a requisite.

Confirmatory Tests

Usually a range of ELISAs based on different formats (e.g. sandwich and antiglobulin) are used: if reactive in two or more, the likelihood of true positivity is very high. A gelatin particle assay for titration can also be very helpful in confirming anti-HIV reactivity. For anti-HIV, Western blot is also often used although in the UK it is not taken as the 'gold standard' and is usually restricted to 'difficult' samples. Because of the widespread experience with anti-HCV RIBA-3 (Ortho Diagnostics) this is the test upon which a serological definition of false-positive, indeterminate and positive is based in the UK. Anti-HCV-reactive samples are also tested by PCR in Scotland but the use of this test is often restricted to indeterminate samples in England. PCR for HIV DNA on a sample containing white blood cells may sometimes be used on 'difficult' samples.

Fate of the Unit

The fate of the unit is determined by the result of the screening ELISA test. Donations which are repeatedly reactive in the screening test are not eligible for transfusion. The management of the donor and subsequent donations from such donors are determined by the results of confirmatory testing. Carefully evaluated and documented procedures have been defined which may allow donors who have on occasion given reactive results in screening tests to be re-instated to the active donor panel on the basis of negativity in a validated alternative ELISA. An essential prerequisite for this approach is that negative results are obtained on confirmatory testing on at least two separate occasions at least 6 months apart. Such approaches would not be applied to indeterminate (as opposed to false-positive) donors who are not considered eligible to donate blood for transfusion.

Management of the Donor

Arrangements are made to obtain further samples to follow-up donors with indeterminate results. The specific approaches currently vary in different centres. Some centres will contact the donor and arrange for additional samples to be taken for further testing, while others will simply 'flag' the donor by computer and undertake additional testing on the subsequent donation. One sensible approach would be to assess the results on an individual basis and to tailor the management to the findings. In the context of HCV testing, for example, a different approach may be appropriate for donors with weak reactivity to NS4 or NS5 antigen, in an immunoblot, as op-

posed to donors whose serum shows strong reactivity to structural core antigens. Where an indeterminate status is confirmed the donor is contacted, and where appropriate and with the permission of the donors, their general practitioner will be informed of the situation. A truly indeterminate donor is not eligible to donate blood for transfusion purposes.

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Question 1

The anti-HCV is performed by ELISA (Abbott). No confirmatory test has been done. To determine the result, we, at first, used the cutoff value with a 10% grey zone. Those within the grey zone were repeated with PCR and the results were consistently negative. After a period of time, we stopped using the grey zone and only the cutoff value is used. Any unit with a positive result will be discarded.

Question 2

The anti-HIV is performed by ELISA (Abbott or Behring). If the result is positive, the test is repeated with two other ELISAs based on different principles. If still positive (all three manufacturers' assays), that unit will be labelled positive. If inconclusive (two out of three), that unit will be confirmed by Western blot. Any unit with indeterminate results will not be used and the donor in question has to stop donating blood for ever.

Question 3

We do not perform a test for anti-HTLV-I/II as the infection has never been observed in Thailand.

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According to the Guidelines of the Blood Transfusion Council of The Netherlands Red Cross [1], all blood donations are screened for the presence of antibodies to HIV-1/2, HCV, HTLV-I/II, *Treponema pallidum* and for the presence of HBsAg. Screening for anti-HIV-1/2, anti-HCV and anti-HTLV-I/II is performed by enzyme linked immunosorbent assays (ELISAs). Upon initially negative ELISA results, the products from the donation may be released, provided other parameters do not interfere. Upon an initially reactive ELISA, the donation and blood products derived thereof are placed 'on hold' ('in isolation'), and the ELISA is repeated in duplicate by the same method as was used for the screening. If one or more of the ELISAs performed in duplicate is reactive, the ELISA is referred to as 'repeatedly reactive' or 'positive'. All blood donations and derived products with such a 'positive' ELISA result are discarded (no matter what results confirmatory testing may yield later). If however both ELISA reactions performed in duplicate are negative, the ELISA is interpreted as 'negative' and the products of the donation may be released from 'isolation', provided other parameters do not interfere. This algorithm is employed for all infectious disease testing of blood donations in The Netherlands.

Confirmatory antibody testing is performed by an external reference laboratory. For the three mentioned viruses, eg. HCV, HIV and HTLV, confirmatory testing is divided into two steps: the first step consists of serological confirmatory testing, and the second step includes a repetition of the serological confirmation plus additional molecular biological testing by polymerase chain reaction (PCR).

Serological confirmatory testing is performed by immunoblot (IB). In addition, HIV-Ag testing is included in serological confirmatory testing for HIV-1/2, since 3rd-generation ELISAs may be more sensitive than Western blot (WB) in early HIV infection. The IB method employed for HIV-1/2 and HTLV-I/II is usually of WB format, enhanced by recombinant or peptide antigens (DBL HIV blot 2.2, DBL HIV-2 blot 1.2, DBL HTLV blot 2.4, Diagnostic Biotechnology, Singapore), and for HCV a recombinant immunoblot is used (RIBATM HCV 3.0 SIA, Chiron, Emeryville, Calif., USA).

If in the first step serological confirmatory testing is negative (for HIV-1/2 both IB and

HIV-Ag should be negative), the ELISA is interpreted as 'false-positive': the donor is not notified and may donate again; the next donation may be released, provided the ELISA is then again negative. If on a next donation such a donor is again ELISA 'false-positive', the donor is notified of the 'false-positive' results on the two occasions and is debarred for 2 years for logistical reasons. The donor may be eligible again after these 2 years, provided the then current regulations are met. The second step of the confirmatory algorithm is to be performed on a separate blood sample, obtained after notification and counselling of the donor, and applies only to donors with either indeterminate or positive serological confirmatory test results in the first step. In addition, at the counselling session such donors are interviewed for risk factors with a nationally standardized questionnaire. Apart from the option that the second step provides for excluding sampling or clerical errors at notification of donors for confirmed positive test results, the second step is most frequently used for decision making on indeterminate IB test results. Indeterminate IB test results could represent limited antibody reactivity during the early phase of, for instance, HIV and HCV infection, limited cross-reactivity with variant virus strains such as in HCV, HIV-2 or HTLV-II infection, low level antibody reactivity in some persistent infections with HCV and HTLV-I, and sometimes late and clearing HCV infection. The vast majority, however, are nonspecific reactivities to single antigens.

It is just after the indeterminate IB findings (of the first step) that PCR becomes important, in most cases to reassure the donor. The standardized assessment of risk factors is of additional help. For donors with indeterminate IB but negative PCR results, the following rule applies: if the ELISA, which is used in the blood bank is again repeatedly positive at a second donation, the donor is to be debarred and may be eligible again after 2 years, provided the then current regulations are met. If, however, the ELISA is negative on the next donation, the donor may be reentered to the donor base and products may be released. For PCR procedures, commercially available methods are often preferred because of reliability and reproducibility [2]. At present, mostly the Roche system is used for HCV-cDNA (Amplicor HCV test with a modified RNA extraction [3], Roche Diagnostic Systems, Branchburg, N.J, USA), HIV-DNA (Amplicor HIV test [4], Roche Diagnostic Systems) and HTLV-I/II-DNA (Amplicor HTLV-I/II test [5], Roche Diagnostic Sys-

tems). For the latter virus, a genotyping step is included in the diagnostic procedure.

At present, the donors (temporarily) deferred for indeterminate IB results largely outnumber those counselled for confirmed positive results. It is therefore important that IBs with high specificity should be used, which can greatly diminish the relatively large number of donors unduly deferred. For instance, in anti-HTLV-I/II testing a more specific and equally sensitive recombinant immunoblot assay is to be preferred over the good old Western blot as the 'gold' standard [6]. Regulatory offices should anticipate this direction and refer to immunoblotting as the technique of choice rather than using one of the four compass points as a prerogative.

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